New, Untrasensitive Technique for the Detection of Organisms and their Biomarkers

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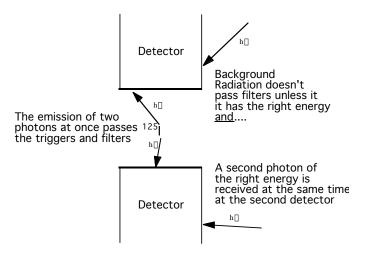


Figure. MultiPhoton Detection

Innovative Claims/NASA Significance

MPD is able to detect radioactivity, and hence radiolabels, at below background levels. The use of MPD allows us to detect hundreds of radiolabels (<10⁻²¹ mole). By radiolabeling proteins, we can use MPD to detect them at well below attomole levels of sensitivity. This is low enough that the detection of, for example, the signature proteins produced by a small number of cells (perhaps even one cell) is potentially feasible.

This technique has a large number of potential applications to problems that span NASA's interests from the physiology of humans in space, to planetary protection, to Mars return sample handling protocols, to the search for life.

Description

The objective of this proposed work will be to apply Multi-Photon Detection (MPD) using radiolabels to demonstrate the detection of proteins at the sub-attomole level. The tasks proposed will focus first on developing procedures to optimize the level of detection using a variety of test proteins. We will then compare the detection of proteins from both live and degraded organisms. MPD will be applied to organisms from each of the domains (Archaea, Bacteria, and Eucarya) and a variety of surface materials (including ceramics, minerals, and metals) that represent a variety of samples such as from spacecraft surfaces to planetary return samples. Finally work will then move from the general detection of proteins to the detection of specific proteins formed in response to environmental changes. Limits of MPD protein sensitivity will be quantified.

Plans

Year 1:

• Work will first establish and optimize procedures to be used for the d of a variety of proteinstochrome C551 fromseudomonasaeruginosaTaq DNA polymerase fromhermusaquaticusand bovine hemoglobin) in solutio

establishing that the method is general for all types of proteins and elir any unexpected problems. General detection limits will be established.

• Work will begin on the general detection of proteins from organisms ir suspension (looking at yeast, from Eutharya, as the first test organism), and withoutlysing agents.

Year 2:

- Work will finish on the detection of proteins from organisms in susper looking at organisms from the domains of BacterArchadea Sulfolobus, and E.coli). Limits for the detection of proteins from organisms, and for th of organisms, will be established.
- Work will begin on detection on different types of samples, starting waluminum, establishing procedures for extracting proteins from those t samples and establishing detection limits.

Year 3

- Work will finish on detection from different types of samples, includin looking at basalt, glass, and materials with cracks and rough surfaces. look at live organisms and the degradation of organismslythingtoheat, freezing, and/or desiccation. Detection limits will be established.
- Work will establish, and set detection limits for, the detection of heat proteins in the response to heat stress by microbes. We will establish I a response to environmental change can be detected.